Although the probe at the 3′ end will be separated from those at the 5′ end of the gene in case of fusion, separation does not specifically target the bcr.

Davies et al. designed a customized *ROS1* break-apart probe set made of clones CTD-2314K7 (chr6: 117,338,338-117,438,446) and RP11-59K17 (chr6: 117,448,944-117,627,275), covering exons 42 and 43 and sequences flanking the 3′ part of the gene, and RP11-623N3 (chr6: 117,654,640-117,833,020) and RP11-117O13 (chr6: 117,830,521-117,971,596) covering sequences flanking the 5′ part and the first 31 exons and a small portion of intron 31 of the gene (Fig. 1).[5] This construct leaves a 27 kb gap in which bcr is located.

These FISH "home-made" probe sets differ not only by the BAC clones used to build them but also by the distance between clones, varying from 27 to 246 kb. In fact, the greater the distance from bcr, the more likely false positives could happen. Indeed, breaks could occur outside *ROS1* bcr and even outside the gene, without fusion leading to kinase activation. Also, it has been shown that deletion of the 5′ region could be associated with a *ROS1* fusion, as it has been reported to happen during fusions of other genes such as those involving *ABL1*, *MLL*.[6] In these cases, deletion occurs at the breakpoint site. Therefore, identifying a deletion of RP11-835I21, as used by Rogers et al.[1] and Bergethon et al.,[3] does not mean that sequences of the 5′ part of *ROS1* were removed.

The diversity in probe design could explain, at least partially, the discrepancies between IHC and FISH results. Home-made probes are a good alternative to commercially available probes but they have to be designed carefully. Interpretation of the results requires a good knowledge of the design of the probes being used to enable mechanisms of the chromosomal and molecular rearrangements to be elucidated.

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In Response:

We thank Uguen et al. for their interest in our recently published manuscript titled Comparison of methods in the detection of *ALK* and *ROS1* rearrangements in lung cancer.[1] We acknowledge their concerns regarding the use of homemade fluorescent in situ hybridization (FISH) probes and the importance of their design. At the time that the study was conducted there were no commercial *ROS1* FISH probes available therefore we used the home-made *ROS1* FISH probe that was kindly gifted to us from Translational Research Laboratory, Massachusetts General Hospital and previously utilized in the study by Bergethon et al.[2] Subsequently, when commercial *ROS1* FISH probes became available, specifically the Cytocell Aquarius *ROS1* Breakapart FISH Probe (Cambridge, UK) and Vysis 6q22 *ROS1* Break Apart FISH Probe Kit (Des Plaines, IL), we repeated the cases which were considered *ROS1* positive or atypical. The Cytocell Aquarius *ROS1* Breakapart FISH Probe showed the same results as with the homemade *ROS1* FISH probe. However, the Vysis 6q22 *ROS1* Break Apart FISH Probe Kit showed the same results for the positive case which contained the break and the atypical case (loss of 3′ end) but the two other cases defined by loss of the 5′ end using the home-made *ROS1* FISH probe were negative by the Vysis 6q22 *ROS1* Break Apart FISH Probe Kit. This as anticipated and highlighted by Uguen et al. show there are differences in FISH signal patterns that is dependent on probe design.

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